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ORIGINAL ARTICLE

Effect of steam treatment for the enzymic saccharification of waste mushroom medium after cultivation of shiitake mushroom (*Lentinula edodes*) and enokitake mushroom (*Flammulina velutipes*)

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Abstract Waste mushroom medium of shiitake and enokitake (WS and WE, respectively) was steamed (StmWS and StmWE, respectively) and saccharized with cellulase to obtain glucose which can be feedstock of bioethanol or other bioproducts. Mushroom medium before cultivation of shiitake and enokitake (MS and ME, respectively) was also steamed (StmMS and StmME, respectively) and saccharized to compare with WS and WE. WS was stored at 15 °C for 1 month (SS), and SS was steamed (StmSS) and saccharized. The amount of glucose from StmSS was more than that from StmWS. The cellulose contained in StmSS and StmWE was saccharified earlier than that in StmMS and StmME, respectively. When StmSS and StmWE-added Meicelase of 5 and 3 FPU/g substrate (9.7 and 5.8 mg Meicelase/g substrate), respectively, was saccharized for 120 h, the saccharification ratio (the amount of glucose saccharized from cellulose/theoretical amount of glucose from cellulose) of them exceeded 80 %. The amount of glucose obtained from StmSS and StmWE was 311.0 and 207.7 mg/g substrate under the condition. It was found that the cellulose contained in mushroom medium was saccharized with less amount of cellulase by the influence of cultivation of shiitake or enokitake, storage of shiitake, and steaming.

Keywords Waste mushroom medium · *Lentinula edodes* · *Flammulina velutipes* · Steam treatment · Enzymic saccharification

Introduction

Shiitake mushroom (*Lentinula edodes*), a white-rot fungus, is one of the most widely produced edible mushrooms in the world [1]. In 1997, the total worldwide production of this mushroom was more than 1,300,000 t and has been increasing ever since [2]. Approximately 82 % of the 75,016-t fresh shiitake harvested in Japan in 2009 was cultivated on synthetic media [3]. The fresh weight of WS is estimated to be in excess of 50,000 t/year in Japan.

Enokitake mushroom (*Flammulina velutipes*), another white-rot fungus, is the most produced and consumed fresh edible mushroom in Japan [3]. The total production and consumption was 138,501 t/year in 2009 [3]. Most commercial enokitake is cultivated on synthetic medium. The fresh weight of WE is estimated to be in excess of 190,000 t/year.

In Japan, MS and ME are mainly composed of hardwood and corncob meal, respectively. Cellulose, hemicellulose, and lignin are contained in WS [4, 5] and WE [6, 7]. Therefore, WS and WE are regarded as lignocellulosic biomasses. As an alternative to fossil resources, lignocellulosic biomass is expected to be used as feedstock for enzymic saccharification to produce bioethanol and other bioproducts because it is a renewable resource. However, lignocellulosic biomass has not been extensively used as feedstock. The major reasons underlying this are the high costs involved in its transportation and storage as well as the lack of an effective pretreatment for enzymic saccharification. The costs of transportation for collecting the

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resource are high because lignocellulosic biomass is bulky and has a low density [8, 9]. High storage costs are incurred because lignocellulosic biomass has seasonal availability [9]. WS and WE are more advantageous than other types of lignocellulosic biomass because of their high density and year-round availability. Shiitake and enokitake are cultivated in mushroom factories in a narrow zone, and a stable amount of their fruiting bodies are harvested every day because synthetic medium cultivation allows them to be produced in a factory. It has been demonstrated that 50–60 % of the cellulose of WS is easily saccharized using an enzyme without pretreatment with acid, alkali, or high heat [4]. Although it is important that a 50–60 % of saccharification ratio was obtained without pretreatment, pretreatment is needed to improve the saccharification ratio of WS.

Mushroom cultivation and storage of maitake (*Grifola frondosa*) [10, 11] and shiitake [4] makes the cellulose in the mushroom medium more susceptible to attack by cellulase. Because enokitake is the same white-rot fungus as maitake and shiitake, there is the possibility that the saccharification ratios of β-glucan of WE and stored WE are high. Therefore, as a preliminary test, we saccharized WE and stored WE as described previously [4]. However, the saccharification ratios of β-glucan were low (<12 %). Therefore, pretreatment is needed to improve the saccharification ratio of β-glucan of WE.

Steam pretreatment is known to improve the enzymic saccharification ratio of hardwood and herbaceous biomass [12]. Because only water is used without alkali, acid, or organic solvent in steam treatment, steam treatment was selected as an ecologically friendly pretreatment to improve the enzymic saccharification ratio of β-glucan of WS and WE. The aim of this study was to verify the improvement in the enzymic saccharification ratio of β-glucan of WS and WE using steam treatment and optimize the conditions of steam treatment and enzymic saccharification.

Materials and methods

Materials

The abbreviated names and outlines of the preparation methods of the substrates used are shown in Table 1. MS was produced using our previous method [4] and autoclaved at 121 °C for 1 h. We produced 27 WS from MS samples according to the previous cultivation method [4]. Eight WS randomly selected samples were stored at 15 °C for 1 month (SS) as described previously [4].

ME and WE were supplied by Hit (Hokkaido, Japan). Before autoclaving, 100 g ME was composed of 35 g corncob meal, 30 g rice bran, 10 g beet pomace powder (all air-dried weights); the remaining 25 g was composed of pH adjuster and other ingredients. Water was added to the mixture. A plastic bottle was filled with the mixture and autoclaved at 121 °C for 1 h (ME). WE was produced from the commercial cultivation of enokitake, Chikumush T-011 (Chikumakasei, Nagano, Japan) with ME. Enokitake inoculated into ME was cultured for 25 days (15 °C, 65–67 % RH). Enokitake fruiting bodies budded for 9 days (14 °C, 92 % RH), were controlled growing for 7–8 days (6 °C, >85 % RH), and were developed for 7 days (8–9 °C, >85 % RH).

Table 1 Abbreviated names of substrates

Substrate name	Explanation of substrate
MS	Mushroom medium before inoculation of shiitake mushroom[a]
WS	Waste mushroom medium after cultivation of shiitake mushroom
SS	Stored WS at 15 °C for a month
StmMS	Steamed MS at 210 °C for 10 min[c]
StmWS	Steamed WS at 210 °C for 3 min[c]
StmSS	Steamed SS at 210 °C for 3 min[c]
ME	Mushroom medium before inoculation of enokitake mushroom[b]
WE	Waste mushroom medium after cultivation of enokitake mushroom
StmME	Steamed ME at 210 °C for 3 min[c]
StmWE	Steamed WE at 210 °C for 3 min[c]

[a] *Lentinula edodes*

[b] *Flammulina velutipes*

[c] The temperature and time conditions of steaming optimized for each substrate in this study

All substrates were oven dried at 50–60 °C until the moisture content became less than 10 %. Dried substrates were milled with a Wiley mill using a 2-mm mesh grating.

Steam treatment

Glass Petri dishes with an inner diameter of 17.9 or 18.5 cm were used for steaming substrates. Dried and milled substrate was put on the Petri dish at 0.09 g/cm^2. Water was added using an atomizer so that the moisture content of the substrate became $\approx$55 % before 1–3 h of the steam treatment. The dish was closed with a stainless steel mesh (80 mesh). The substrate was steamed at 200 or 210 °C (1.56 or 1.91 MPa) using an autoclave (body capacity: 0.767 m^3; Hitachi Zosen, Osaka, Japan). The heat-up and cool-down time for 1.56 MPa was 30–50 and 90–120 s, respectively. The heat-up and cool-down time for 1.91 MPa was 90–120 and 120–150 s, respectively. Steamed substrate was air dried for 2–4 days. The dry matter (DM) of the substrate was weighed before and after steam treatment. DM was calculated by drying a subsample

of the substrate in an oven at 105 °C for 24 h and weighing within an accuracy of ±0.1 mg.

The yield of the steam treatment (Y) was calculated using Eq. 1:

$$Y\ (\%) = A/B \times 100 \quad (1)$$

where, A is the DM of the substrate after steam treatment (g), and B is the DM of the substrate before steam treatment (g).

Chemical components analysis

The chemical components of the substrates were analyzed according to our previous method [4]. However, the content of α-glucan (glucan that can be saccharized by α-amylase and glucoamylase) in WS, StmWS, SS, StmSS, WE, and StmWE was not measured because our previous study [4] and preliminary measurements indicated that the α-glucan content was negligible. β-Glucan contents were calculated by subtracting the α-glucan content from total glucan. When the β-glucan contents in WS, StmWS, SS, StmSS, WE, and StmWE were calculated, the α-glucan contents were assumed to be zero. The total galactan, arabinan, and mannan contents were also designated minor sugars as in our previous report [4].

Enzymic saccharification

Substrates steamed under various conditions were saccharized using a commercial cellulase preparation (Meicelase; Meiji Seika, Tokyo, Japan) according to our previous method [4]. Meicelase (4.8 FPU) and 12 ml 0.1 M sodium acetate buffer (pH 4.8) were added to a substrate (240 mg DM) in a tube at 20 FPU/g substrate. The tubes were subsequently incubated at 40 °C for 24 h on a shaker at 80 rpm.

The enzymic saccharification of StmMS, StmSS, StmME, and StmWE was examined with different amounts of Meicelase and incubation times. StmMS and StmSS (240 mg DM) were placed into a tube, followed by the addition of Meicelase (0.48, 1.2, or 4.8 FPU), 0.24 mg sodium azide, and 12 ml buffer (2, 5, or 20 FPU/g substrate). StmME and StmWE (240 mg DM) were placed into a tube, followed by the addition of Meicelase (0.24 or 0.72 FPU), 0.24 mg sodium azide and 12 ml buffer (1 or 3 FPU/g substrate). These 4 substrates were saccharized for 72 or 120 h. For 72-h incubation, 0.5 ml solution was sampled at 24, 48, and 72 h; for 120-h incubation, 0.5 ml solution was sampled at 72, 96, and 120 h. The amount of saccharized glucose and xylose was analyzed using HPLC as described previously [4].

The saccharification ratio of β-glucan (S_g) was calculated using Eq. 2:

$$S_g\ (\%) = [\{D - (E \times F/100)\}/C] \times 100 \quad (2)$$

where, C is the β-glucan content (mg), D is the amount of glucan hydrolyzed by Meicelase (mg), E is the α-glucan content (mg), and F is the percentage of a ratio of glucose saccharized from starch by Meicelase against theoretical amount of glucose from starch (%) as described previously [4].

The saccharification ratio of xylan (S_x) was calculated using Eq. 3:

$$S_x\ (\%) = G/H \times 100 \quad (3)$$

where, G is the amount of xylan hydrolyzed to xylose by Meicelase (mg), H is the xylan content (mg).

Statistical analysis

The correlation between steaming time and substrate weight was assessed using Spearman's correlation coefficient with rank test. The homoscedasticity of the amount of glucose saccharized from StmWS and StmSS was assessed using the F test. The homoscedasticity of the chemical components and S_g and S_x of each substrate was assessed using Bartlett's test. The difference between the substrates whose homoscedasticity was assumable was examined by Student's t test, one-way analysis of variance (ANOVA), and Tukey–Kramer test. The difference between substrates whose homoscedasticity was not assumable was examined using Games–Howell test. $P < 0.05$ was considered statistically significant.

Results and discussion

Optimization of steam treatment

Table 2 shows the yield of steam treatment (Y) according to steaming time. There was a negative correlation between the steaming time and substrate dry weight in all substrates ($P < 0.05$). Therefore, to optimize the steaming conditions, we determined the condition under which the greatest amount of saccharized glucose was produced according to the substrate dry weight before, rather than after, steaming. MS, WS, and SS were steamed at 190–210 °C for 3–15 min as preliminary tests according to the results of previous studies regarding the steaming treatment of lignocellulose [12–14]. To compare the effects of steaming, each steamed substrate was saccharized with Meicelase (data not shown). In this temperature range, the maximum of the saccharized glucose was more at a higher steaming temperature (210 > 200 > 190 °C). According to these preliminary results, MS, WS, and SS were steamed at 210 °C and saccharized with Meicelase (Fig. 1). The steaming conditions used in the following examinations

Table 2 Changes in substrate dry weight after steaming

Substrate[a]	Steaming temperature (°C)	Changes in dry weight [% (w/w)][b]					
		Steaming time (min)					
		1	2	3	5	10	15
MS	210	–	96.3 ± 0.4	92.8 ± 1.2	87.7 ± 2.0	82.0 ± 0.2	76.9 ± 0.2
WS	210	92.8 ± 0.7	88.5 ± 0.4	86.0 ± 0.8	80.4 ± 0.7	–	–
SS	210	91.7 ± 2.7	88.0 ± 1.1	88.0 ± 1.7	82.0 ± 0.8	–	–
ME	200	–	92.5 ± 0.6	91.0 ± 0.5	86.2 ± 0.9	79.0 ± 0.4	77.0 ± 0.4
WE	210	–	88.9 ± 0.4	80.6 ± 0.7	76.6 ± 0.3	–	–
WE	210	–	–	–	88.1 ± 0.7	77.9 ± 0.7	74.9 ± 0.1

Values represent the mean ± SD of 3 replicates

[a] See Table 1

[b] Percentage of substrate dry weight after steaming relative to that before steaming

were selected on the basis of the mean value. MS, which was steamed at 210 °C for 10 min (StmMS), and WS and SS, which were steamed at 210 °C for 3 min (StmWS and StmSS, respectively), were used in the following analysis. StmSS had significantly more glucose than StmWS ($P < 0.05$). Therefore, StmSS was used in the following enzymic saccharification examinations. Because the production lots of mushroom media between steamed and non-steamed substrates are different, the amounts of glucose gained between steamed conditions could not be compared easily. However, it appears that the amounts of glucose from MS, WS, and SS steamed in various conditions were greater than those from their non-steamed counterparts.

ME and WE were steamed at 180–210 °C for 3–15 min as preliminary tests according to the results of previous studies regarding the steaming treatment of lignocellulose [12–14]. Each steamed substrate was saccharized with Meicelase (data not shown). In this temperature range, the maximum of saccharized glucose was more at a higher temperature as with MS, WS, and SS. However, the difference in the amount of glucose from WE steamed at between 200 and 210 °C in the preliminary tests was not clear. Therefore, ME was steamed at 210 °C, and WE was steamed at 200 and 210 °C according to these preliminary results (Fig. 2). With steam or hot water treatment as pretreatment for the enzymic saccharification of lignocellulose, it is known that extending the treatment time increases the amount of glucose per gram of treated substrate [12–14]. Meanwhile, it is also known that excess treatment time decreases substrate weight and the amount of glucose per gram of substrate before treatment [12–14]. Both upward and downward trends were observed in WE at 200 °C between 5 and 15 min and at 210 °C between 2 and 5 min. Therefore, the other steaming time for WE was not examined. The steaming condition used in the following examinations was selected on the basis of the mean value. ME and WE, which were steamed at 210 °C for 3 min,

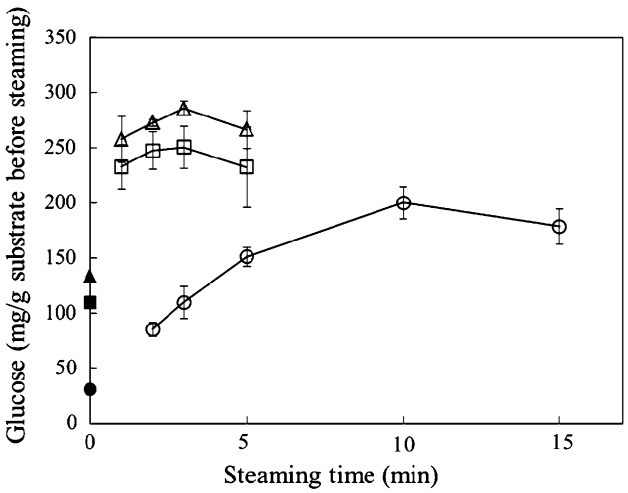

Fig. 1 Optimization of steaming conditions for the enzymic saccharification of MS, WS, and SS using Meicelase (20 FPU/g substrate) at 40 °C for 24 h ($n = 3$). *Open symbols* the mean values of the amounts of saccharized glucose from the substrates steamed at 210 °C. *Closed symbols*, the mean values of the amounts of saccharized glucose calculated on the basis of the data of non-steamed substrates of Hiyama et al. [4]. *Circles*, *squares*, and *triangles* represent MS, WS, and SS, respectively. *Error bars* standard deviations

were used in the subsequent analysis and enzymic saccharification. The amounts of saccharized glucose from non-steamed ME, WE, and stored WE were <40 mg/g substrate (preliminary test, data not shown); those from ME and WE, which were steamed at 200 or 210 °C for 2–15 min, were >124 mg/g substrate before steaming under the same enzymic saccharification condition.

Chemical components

The lignin and sugar contents of selected substrates were analyzed (Table 3). Acid-insoluble lignin content after steaming was significantly greater than that before steaming in all substrates. Xylan content after steaming was

significantly lesser than that before steaming. There were no significant differences in β-glucan content before and after steaming except for that in MS and WS. Although the β-glucan contents in StmMS and StmWS were significantly greater than those in MS and WS, respectively, the ratios of the changes seemed to be smaller than those for lignin and xylan.

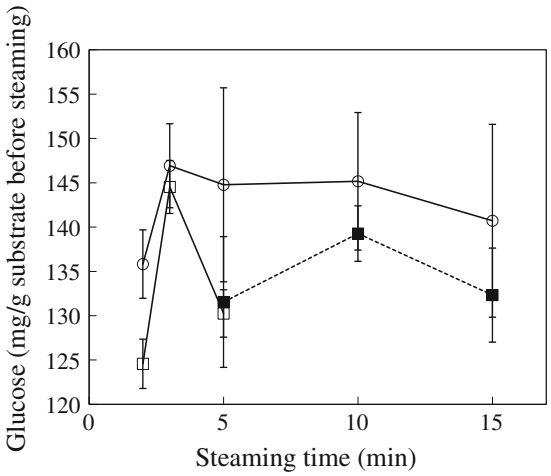

Fig. 2 Optimization of steaming conditions for the enzymic saccharification of ME and WE using Meicelase (20 FPU/g substrate) at 40 °C for 24 h ($n = 3$). *Open and closed symbols* the mean values of the amounts of saccharized glucose from the substrates steamed at 210 and 200 °C, respectively. *Circles* and *squares* represent ME and WE, respectively. *Error bars* standard deviations

Although the acid-insoluble lignin contents in WS and SS were significantly smaller than that in MS, the difference between ME and WE was not significant. Similarly, although the xylan contents in WS and SS were significantly lesser than that in MS, there was no significant difference between ME and WE. The degradation of acid-insoluble lignin and xylan in mushroom media lignocellulose by enokiitake cultivation was less than that by shiitake cultivation.

The β-glucan content in MS was significantly greater than that in ME. The lignocellulose contents in mushroom medium of shiitake and enokitake were >60 and 45 %, respectively. After cultivation or storage, the β-glucan contents in WS, SS, StmWS, and StmSS were significantly greater than those in WE and StmWE. Therefore, the results indicate that WS is superior to WE as a glucose feedstock.

Enzymic saccharification

The changes in the S_g values of StmMS and StmSS when the amount of Meicelase was decreased are shown in Fig. 3a. When the amount of Meicelase added was the same, the S_g values of StmSS was significantly higher than that of StmMS under all saccharification conditions except 20 FPU/g substrate addition at 96 and 120 h saccharification time. The partial degradation of lignocellulose in

Table 3 Lignin and sugar contents obtained from the substrates and dry weight changes

Substrate[a]	Component [% (w/w)][b]							Changes in dry weight [% (w/w-MS[e] or ME[f])]
	Acid-insoluble lignin	Acid-soluble lignin	β-Glucan	Xylan	Minor Sugars[c]	α-Glucan	Other components[d]	
MS	24.8 ± 0.5 de	3.3 ± 0.5 a	28.5 ± 1.8 cd	13.7 ± 0.6 i	6.2 ± 0.2 abc	4.9 ± 0.5	l8.7 ± 1.5	100.0 ± 1.1
StmMS	39.0 ± 1.5 g	4.6 ± 0.2 b	33.6 ± 1.8 e	4.7 ± 0.9 a	4.4 ± 0.6 a	2.4 ± 0.2	11.3 ± 1.1	82.0 ± 0.2
WS	13.8 ± 0.4 ab	6.4 ± 0.1 cd	26.2 ± 1.8 bc	9.5 ± 0.5 def	10.1 ± 0.8 e	–	34.1 ± 2.4	45.0 ± 0.4
StmWS	27.0 ± 0.1 e	6.1 ± 0.1 c	34.4 ± 3.1 e	6.9 ± 0.7 bc	6.3 ± 1.3 abc	–	19.2 ± 2.5	38.7 ± 0.7
SS	11.8 ± 0.4 a	6.9 ± 0.3 de	33.7 ± 3.0 e	11.5 ± 1.2 fgh	8.8 ± 1.2 de	–	27.3 ± 3.5	40.2 ± 0.9
StmSS	23.5 ± 2.4 d	6.4 ± 0.4 cd	32.1 ± 0.2 de	6.2 ± 0.8 ab	8.5 ± 0.1 de	–	23.4 ± 1.7	35.2 ± 1.4
ME	16.6 ± 0.9 bc	6.9 ± 0.1 de	19.3 ± 1.0 a	11.6 ± 0.9 fghi	7.2 ± 0.4 cd	6.1 ± 0.5	32.4 ± 1.2	100.0 ± 2.3
StmME	26.4 ± 1.0 de	7.6 ± 0.1 ef	18.3 ± 1.2 a	9.0 ± 0.2 cd	6.4 ± 0.4 bc	6.7 ± 0.4	25.6 ± 1.3	91.0 ± 0.5
WE	19.6 ± 0.4 bc	6.9 ± 0.2 de	18.5 ± 0.4 a	12.9 ± 0.6 ghi	6.0 ± 0.5 abc	–	36.2 ± 0.8	52.8 ± 3.7
StmWE	30.8 ± 0.9 f	7.7 ± 0.2 f	22.5 ± 1.0 ab	9.3 ± 0.2 de	4.7 ± 0.1 ab	–	24.9 ± 1.9	43.3 ± 0.1

Values represent means ± SD of 3 replicates except for changes in dry weight of WE. The value of change in the dry weight of WE represents the mean ± SD of 31 replicates

Numbers with different letters indicate significant differences between substrates ($P < 0.05$) according to the Tukey–Kramer test

[a] See Table 1

[b] Percentage of the weights of lignin and sugars relative to the dry weight of each substrate

[c] Total galactan, arabinan, and mannan contents

[d] Dry weight minus the sum of acid-insoluble and acid-soluble lignin, β-glucan, xylan, minor sugars, and α-glucan

[e] Percentage of the dry weights of StmMS, WS, StmWS, SS, and StmSS after treatment (shown in Table 1) relative to the dry weight of MS

[f] Percentage of the dry weights of StmME, WE, and StmWE after treatment (shown in Table 1) relative to the dry weight of ME

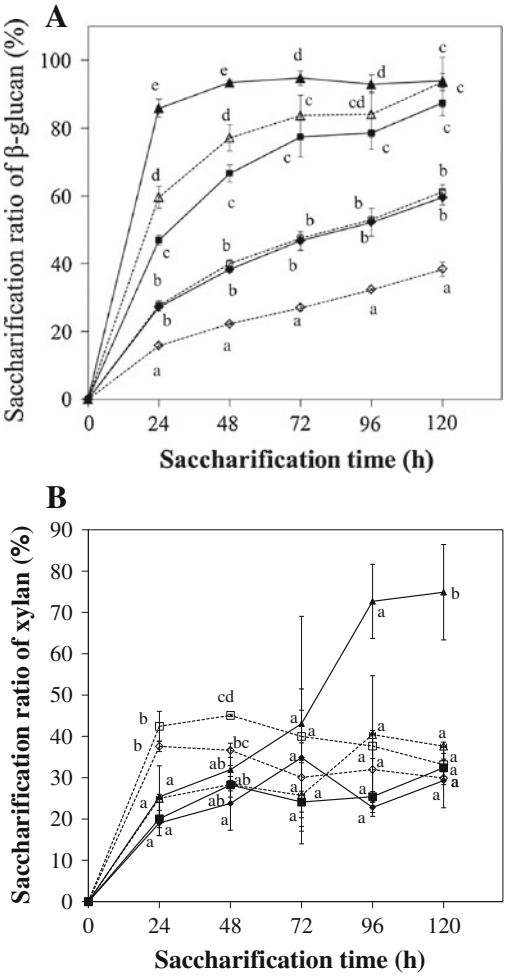

Fig. 3 Time-course changes in the saccharification ratio of **a** *β*-glucan and **b** xylan in StmSS and StmMS with various amounts of Meicelase ($n = 3$ except for 72 h; $n = 6$ for 72 h). *Closed* and *open symbols* the mean saccharification ratios of StmSS and StmMS, respectively. *Triangles*, *squares*, and *diamonds* represent the amount of added Meicelase: 20, 5, and 2 FPU/g substrate, respectively. *Error bars* standard deviations. The significance of difference in saccharification ratios of *β*-glucan and xylan among the substrates and the amounts of Meicelase at the same saccharification time were examined using the Tukey–Kramer test except for xylan at 72 and 96 h. The significance of differences in saccharification ratios of xylan among the substrates and the amounts of Meicelase at 72 and 96 h were examined using the Games–Howell test. *Symbols with different letters* indicate significant differences among substrates and amounts of Meicelase at the same saccharification time ($P < 0.05$)

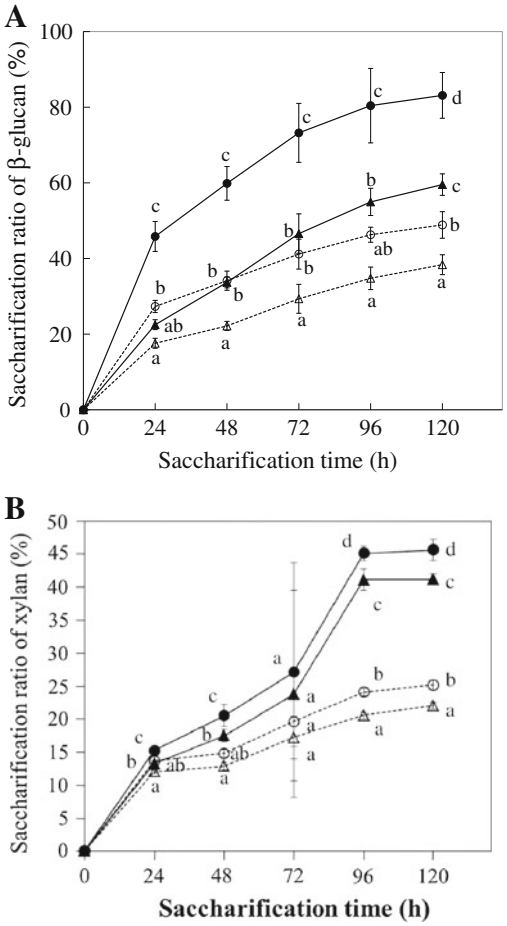

Fig. 4 Time-course changes in saccharification ratio of **a** *β*-glucan and **b** xylan of StmWE and StmME with various amounts of Meicelase ($n = 3$ except for 72 h; $n = 6$ for 72 h). *Closed* and *open symbols* the mean saccharification ratios of StmWE and StmME, respectively. *Circles* and *triangles* the amounts of added Meicelase: 3 and 1 FPU/g substrate, respectively. *Error bars* standard deviations. The significance of differences among the substrates and amounts of Meicelase at the same saccharification time were examined using the Tukey–Kramer test. *Symbols with different letters* indicate significant differences among substrates and amounts of Meicelase at the same saccharification time ($P < 0.05$)

mushroom medium by shiitake mycelia increased its saccharification ratio [4]. The effect remained after these substrates were steamed. The S_g values of StmSS with 20 FPU/g substrate addition at 24, 48, and 72 h was significantly higher than that of the rest. The increase in the S_g value of StmSS with 20 FPU/g substrate addition stopped from 48–72 h and exceeded 90 % from 48–120 h. The S_g value of StmMS with 20 FPU/g substrate addition continued increasing until 120 h; no significant difference between StmSS and StmMS was detected at 96 or 120 h. The S_g value of StmSS with 5 FPU/g substrate addition was 87.3 % at 120 h; no significant difference between StmSS with 20 and 5 FPU/g substrate addition was detected. Although the S_g values of StmSS with 2 FPU/g substrate addition and StmMS with 5 and 2 FPU/g substrate additions were not high (<60 %), they continued increasing until 120 h. The amounts of glucose from StmSS with 20 FPU/g substrate addition at 72 h were 337.1 ± 7.8 and 295.3 ± 7.7 mg/g substrate after and before steaming, respectively.

The changes in the S_x values of StmMS and StmSS when the amount of Meicelase was decreased are shown in Fig. 3b. When StmSS was saccharized with 20 FPU/g

substrate for 96–120 h, S_x exceeded 70 %; however, S_x was less than 50 % in the other condition. There was no clear relation between S_g and S_x, except that both the S_g and S_x values of StmSS with 20 FPU/g substrate were higher.

When the amount of Meicelase decreased, the S_g values of StmME and StmWE also decreased (Fig. 4a). When the amount of Meicelase added was the same, the S_g value of StmWE was significantly higher than that of StmME under all saccharification conditions except with the addition of 1 FPU/g substrate at 24 h. The cultivation of both enokitake and shiitake improved the S_g values of steamed substrates. The S_g value of StmWE with 3 FPU/g substrate addition exceeded 80 % at 120 h. Under this condition, the amounts of glucose per substrate after and before steaming were 207.7 ± 6.8 and 170.3 ± 5.3 mg/g substrate, respectively. Although the S_g values of StmWE with 1 FPU/g substrate addition and StmME with 3 and 1 FPU/g substrate addition were not high (<60 %), they continued to increase until 120 h.

The changes in the S_x values of StmME and StmWE when the amount of Meicelase was decreased are shown in Fig. 4b. S_x was less than 50 % under these conditions. However, there was a clear relation between S_x and S_g; S_x was higher in the saccharification condition in which S_g was higher. It is known that Meicelase has a relatively low xylanase activity [6, 15]. Meanwhile, it is known that removing xylan increases S_g [16, 17]. Therefore, the addition of xylanase may increase S_g or accelerate its rate of change.

The results of this study can be summarized as follows. Steam treatment increases the S_g values of WS and WE. Since the amount of saccharized glucose from StmSS is greater than that from StmWS, this indicates that the effect of storage remains after steaming. The S_g values of StmSS and StmWE exceeded 80 % with the addition of 5 and 3 FPU/g substrate (9.7 and 5.8 mg Meicelase/g substrate, respectively). Few studies have aimed to reduce the amount of additional cellulase required when using WS and WE; for example, WS and WE have been saccharized with 100 and 250 mg Meicelase/g substrate [5, 6]. The results of the saccharification experiment using small amounts of additional cellulase can provide information that will help reduce costs and move toward the practical realization of conversion to glucose from waste mushroom media.

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